A MULTI-OMICS APPROACH TO INVESTIGATE THE PLASMA PROTEOME AND DETERMINE THE MECHANISITIC PROCESSES INVOLVED IN DIFFERENT RESPIRATORY DISEASE CONDITIONS

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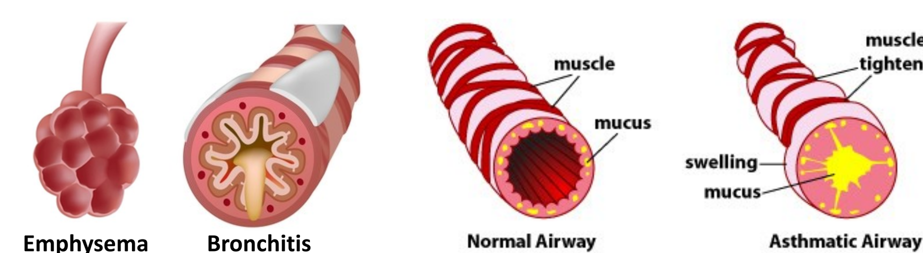
INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the name for a group of lung conditions that cause breathing difficulties. It includes emphysema, which is damage to the air sacs within the lungs, and chronic bronchitis, which is the long-term inflammation of the airways. COPD is a common condition that mainly affects older members of the population, with many having a history of smoking. Asthma is a common lung condition, causing occasional breathing difficulties. It affects people of all ages and often starts in childhood, although it can also develop for the first time in adulthood. Here, we have utilized lipidomic, metabolomic and proteomic approaches, employing the same LC configuration, to reveal factors that may be involved in these biological processes.

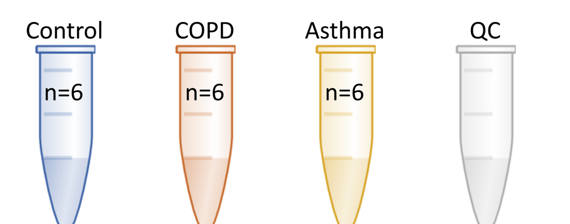


METHODS

Metabolomics samples: Protein precipitation using MeCN & diluted with H_2O

Lipidomics samples: Lipid extraction and protein precipitation using IPA

Proteomic samples: Tryptically digested, undepleted human plasma spiked with Biognosys PQ500 SIL peptides.



Injected samples in all analyses consisted of controls (n=6, mixed), chronic obstructive pulmonary disease (n=6, mixed) and asthma (n=6, mixed). In addition, a pool of all conditions/ samples acted as a QC.

LC conditions

An Acquity I Class was configured for the three experiments as described in **Table 1**.

Experiment	Column	Flow Rate (µL/min)	Composition change	Gradient time (min)
Metabolomics	BEH amide 2.1x100mm	700	100 to 50% 5:95 H_2O:MeCN w/ FA and ammonium formate	10
Lipidomics	CSH C18 2.1x100mm	400	3 to 40% IPA:MeCN w/ ammonium formate	20
Proteomics	CSH130 C18 1x100mm	50	1 to 40% MeCN (+0.1% formic acid)	15, 30 or 45

***Table 1**. LC Configuration and Conditions*

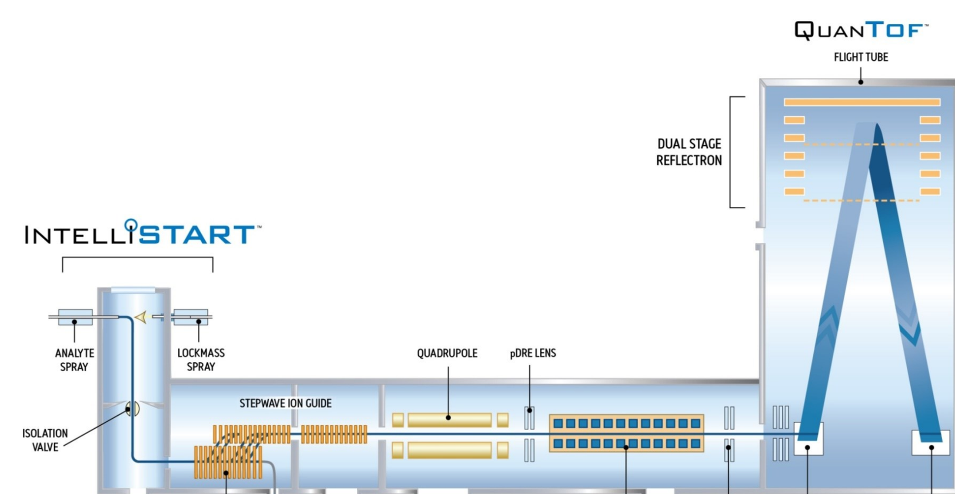


***Figure 1**. Xevo G2-XS QToF Mass Spectrometer*

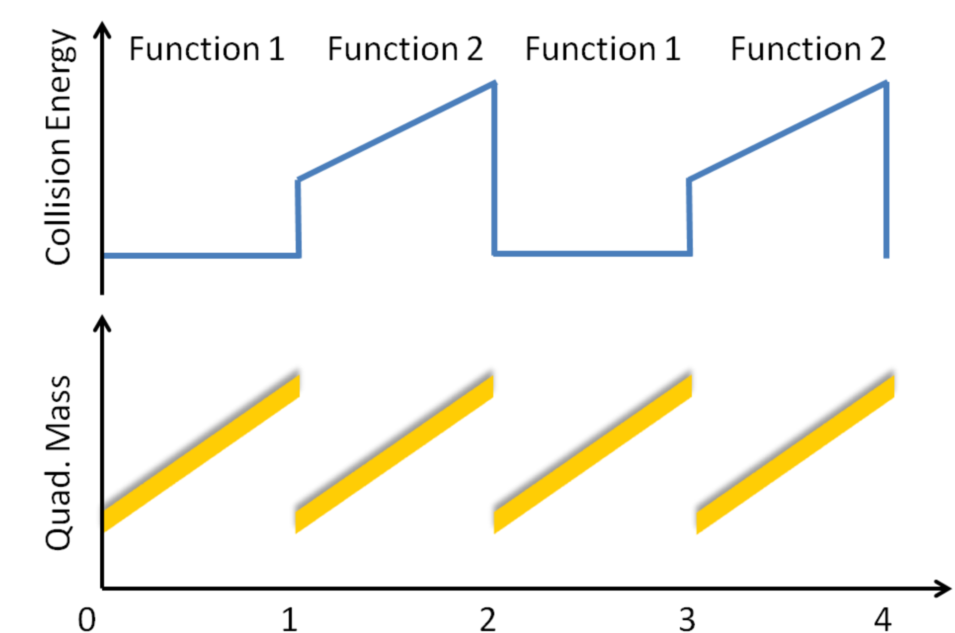


***Figure 2**. SONAR Acquisition Principle*

SONAR[1,2,3] DIA Acquisition Principle (Figures 1 and 2)

The quadrupole of the Mass Spectrometer is programmed to scan over a specific mass range and set to transmit a mass window of between 1 and 50Da. The ToF usually acquires over a different mass range with alternate low and elevated energy scans performed. The low energy scan provides precursor information and the elevated energy scan provides corresponding fragment information. The optimised parameters for each application are summarised in **Table 2**.

Experiment	Quad Scan (Da)	Quad Window (Da)	ToF Scan (Da)	Scan time/fn (s)	Low Energy CE (V)	Elevated Energy CE (V)
Metabolomics	50-600	10	50-1200	0.1	6	20-30
Lipidomics	300-950	10	50-1200	0.1	6	20-40
Proteomics	400-900	20	50-2000	0.5	6	16-36

***Table 2**. Mass Spec SONAR Acquisition Parameters*

Data Processing

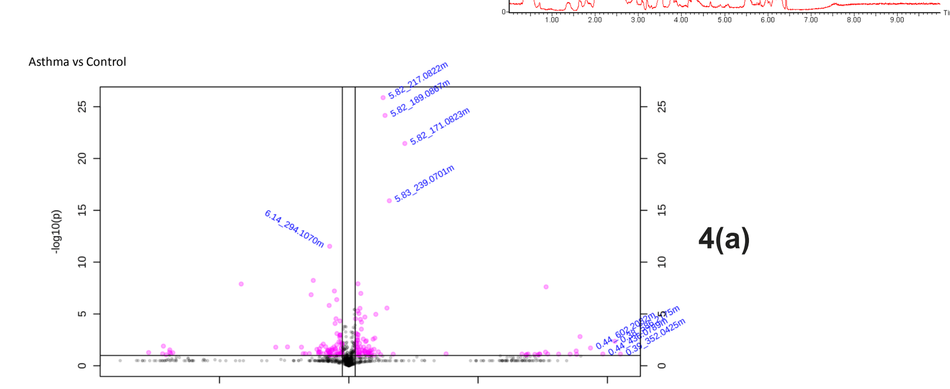
SONAR DIA data were processed using Progenesis QI, Progenesis™ QI for proteomics and Spectronaut™ Pulsar X (Biognosys, Schlieren, Switzerland). Searches were performed using carbamidomethyl C (fixed) and oxidation of methionine (variable) modifications in addition to a 1% FDR. PCA Analysis was through EZInfo (Umetrics, Umea) and Pathway analysis was conducted using Metacore (Thomson Reuters, London)

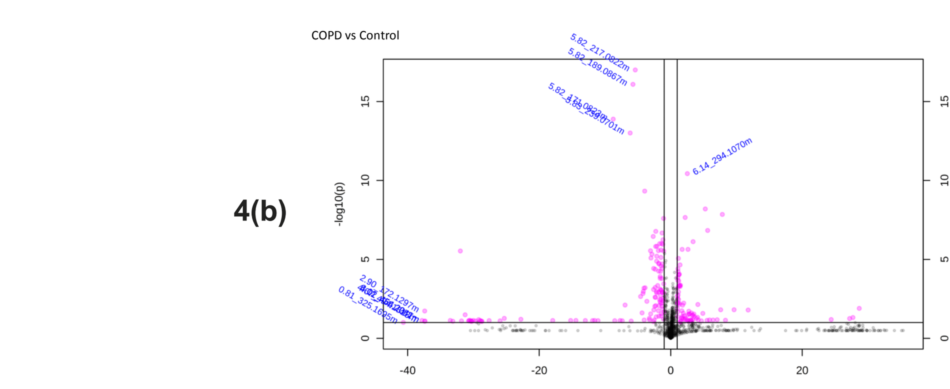
RESULTS

Plasma metabolomics/lipidomics

A combination of both RP and HILIC chromatography were used for the analysis of the metabolite/lipid extracts, **Figure 3**. Following data processing, the normalised data were further interrogated to derive statistically relevant features as shown in **Figures 4** and **5**. Database searching the most significant features identified a variety of molecular classes.

***Figure 3**. Representative chromatogram for HILIC-based separation of polar metabolites extracted from COPD plasma.*





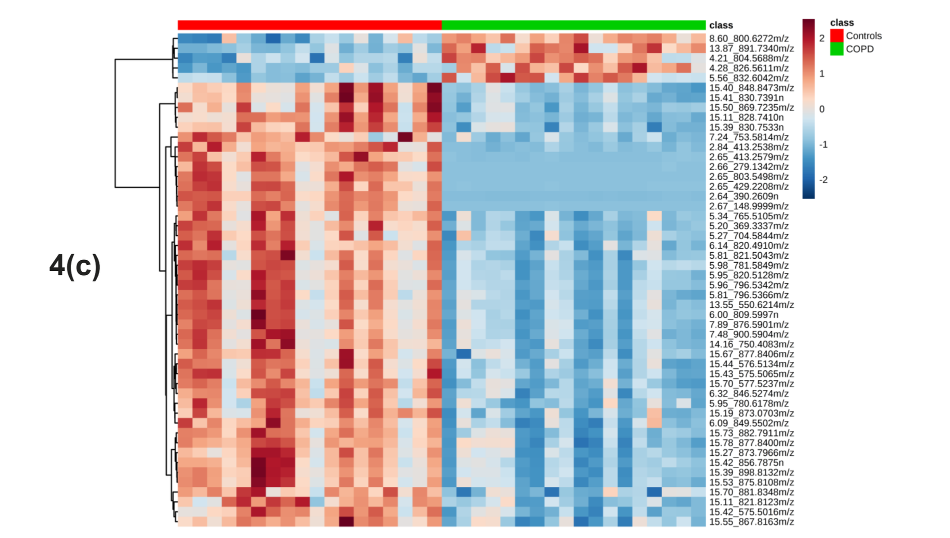


***Figure 4**. Statistical interrogation of the processed data. (a,b)Volcano plots highlighting differential features for metabolites of high statistical significance (fold change >2; ANOVA <0.05) for both Asthmatic (upper) and COPD (lower) patients versus control subjects are shown. (c) The heatmap highlights the top 50 differential lipids between COPD and control subjects based on ANOVA/T-test <0.05.*

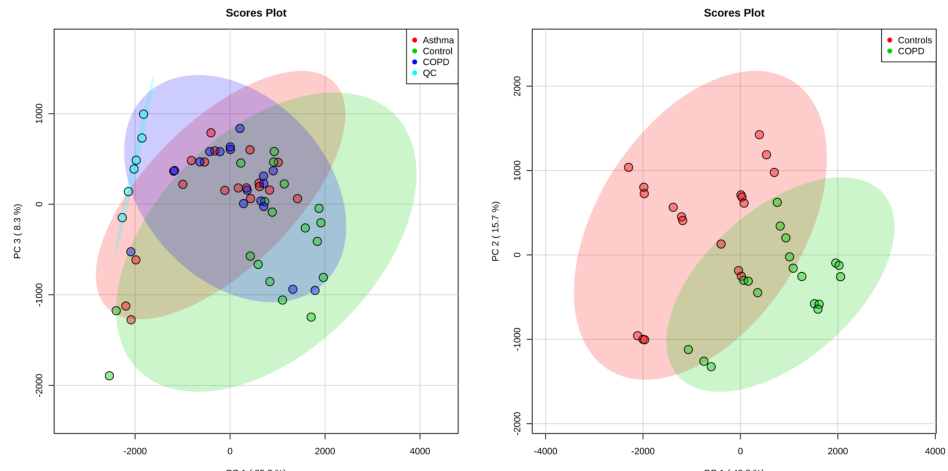
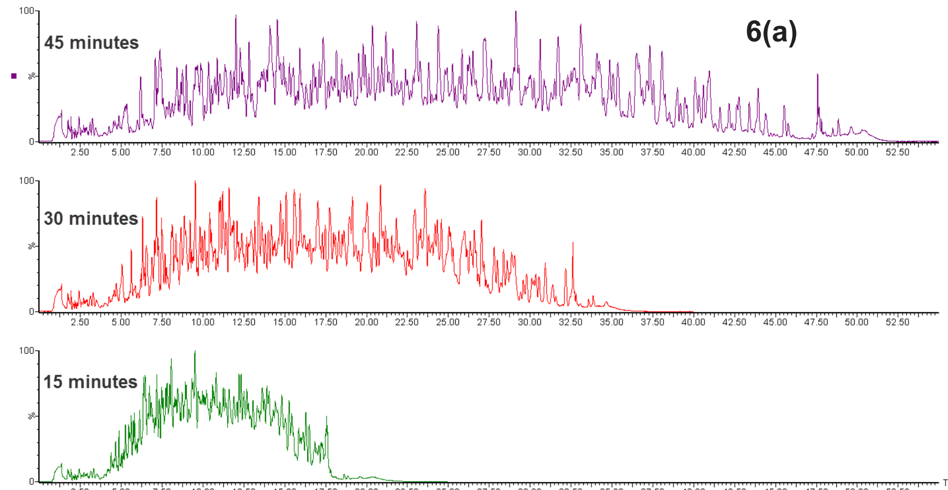


***Figure 5**. Principal Components Analysis plots for metabolites (left, COPD, Asthma, Control and QC) and lipids (right, COPD vs Control). Data shows a separation between the specific disease states compared with control and / or QC.*

Plasma proteomics with 1mm scale chromatography

Proteomics experiments normally involve very low sample amounts and require nanoscale chromatography to provide ultimate sensitivity. However, since samples analysed in plasma proteomics tend to have tryptic peptides present in relatively large amounts, we demonstrate the use of 1mm scale chromatography for these sample types.



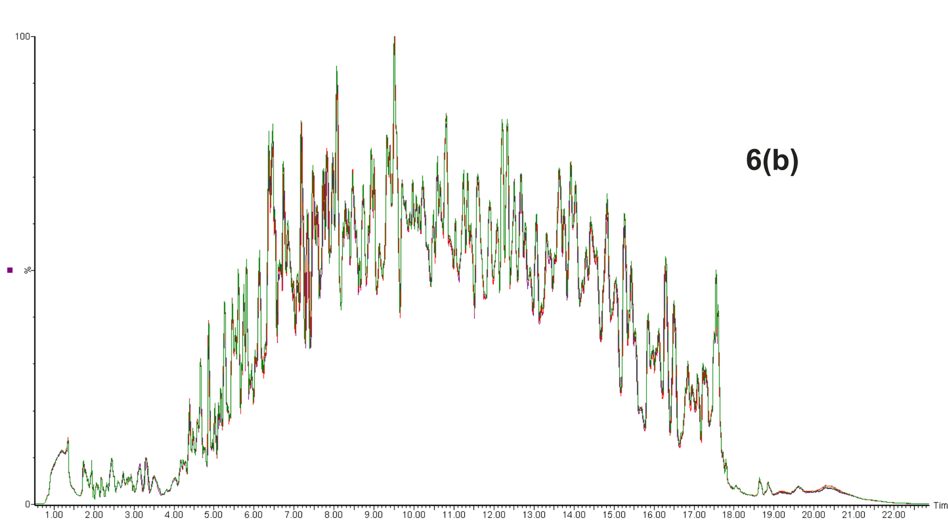


***Figure 6**. Peptide Separation Chromatograms*

Chromatograms in **Figure 6 (a)** represent the effect of gradient length on peptide separation, where shown are data from the three different gradient lengths and a load of 10ug. The excellent chromatographic reproducibility obtained for the shortest gradient length is shown in **Figure 6 (b)**, which represents three overlaid chromatograms for 15min gradient length and a 10ug load.

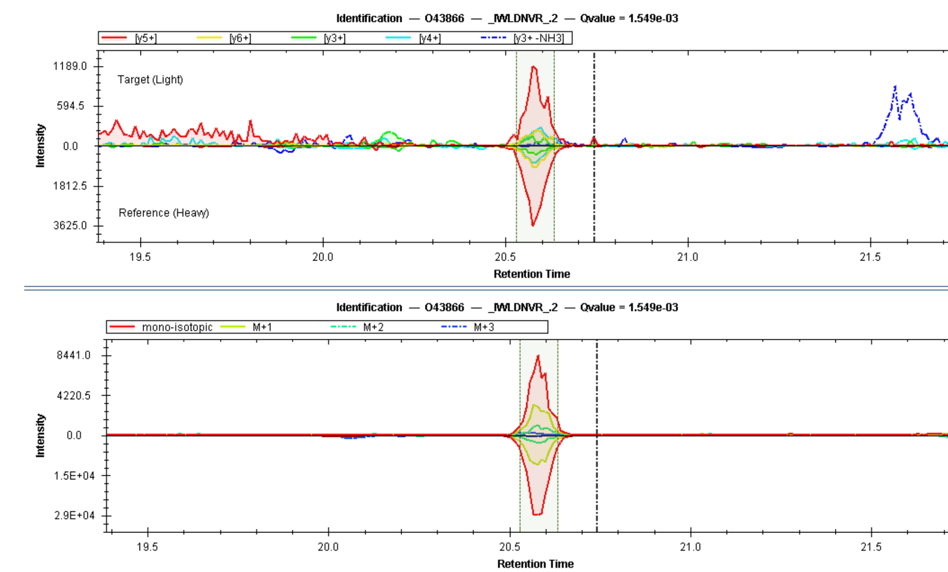


***Figure 7**. Biognosys Spectronaut Representation for Identified Heavy / Light Pair*

Quantitative proteomics often incorporates the use of stable isotope labelled (SILs) peptides spiked into the analyte in order to provide absolute quantitation. For use in plasma proteomics, there is a panel set of more than 500 SIL peptides, Biognosys PQ500. When data is processed using Spectronaut, heavy and light analogues are identified from the peptide panel. For a 10ug load and 45minute gradient, one such heavy and light identification is shown in **Figure 7** for the peptide IWLDNVR from O43866, CD5L_Human. In total, 159 light/heavy pairs were identified in this experiment.

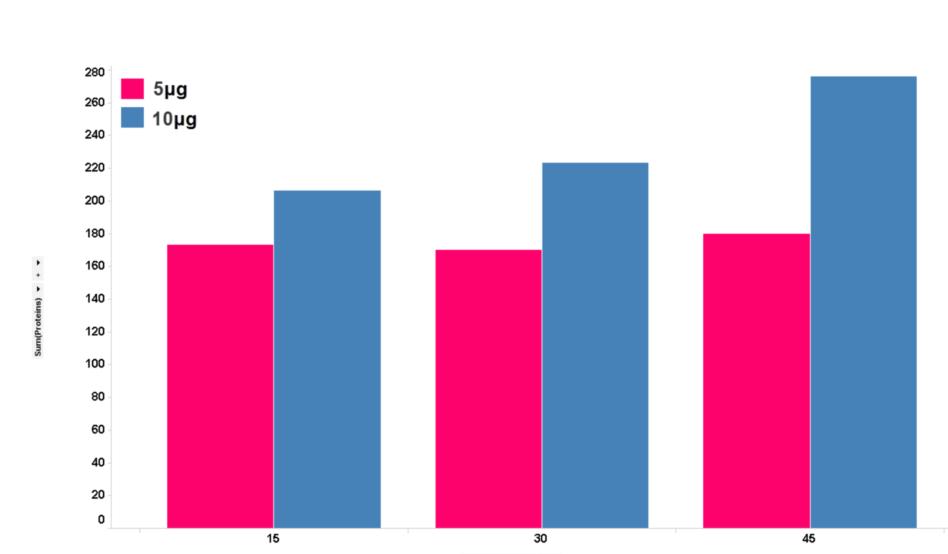


***Figure 8**. Protein IDs (PQIP)*

The total number of quantified protein identifications from searches performed within Progenesis QI.P are shown in **Figure 8**. Gradient length had little effect on protein IDs for the 5ug loadings but longer gradients were more beneficial for the 10ug loading.

The samples used for this study consisted of two different respiratory disease states, normal control and a pooled QC. The PCA plot shown in **Figure 9** indicates that separation of the different groups is readily achieved and corresponding CVs of <8%, **Figure 10**, further demonstrate the technical reproducibility of the data.

The most relevant features from all analyses were subjected to pathway analysis in order to determine the physiological roles/processes implicated in these respiratory conditions. A number of pathways were identified as being potentially implicated in the pathogenesis of both COPD and asthma **Figure 11**.

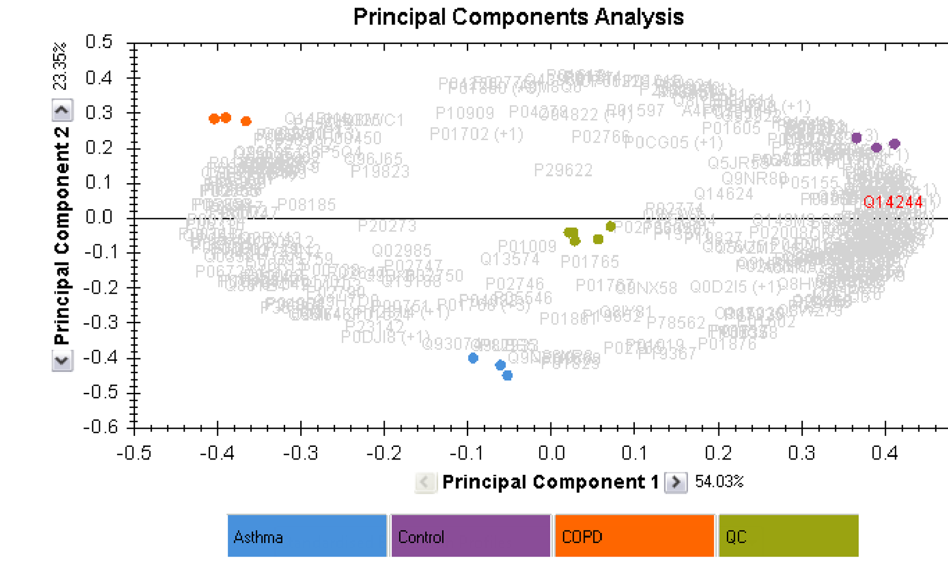


***Figure 9**. PCA Plot from Peptide Measurements showing clear separation of the samples*

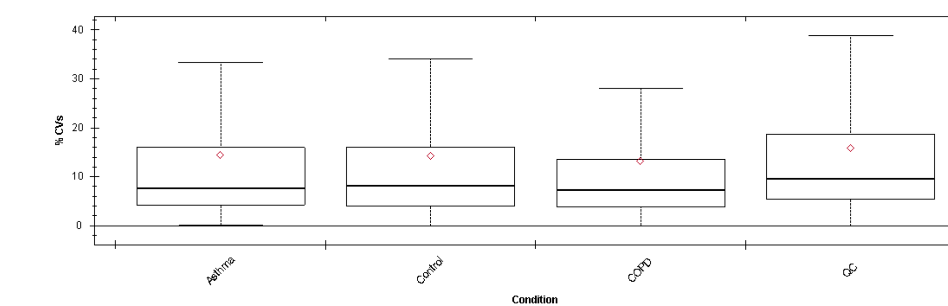


***Figure 10**. CV Measurements for Peptides Analysis*

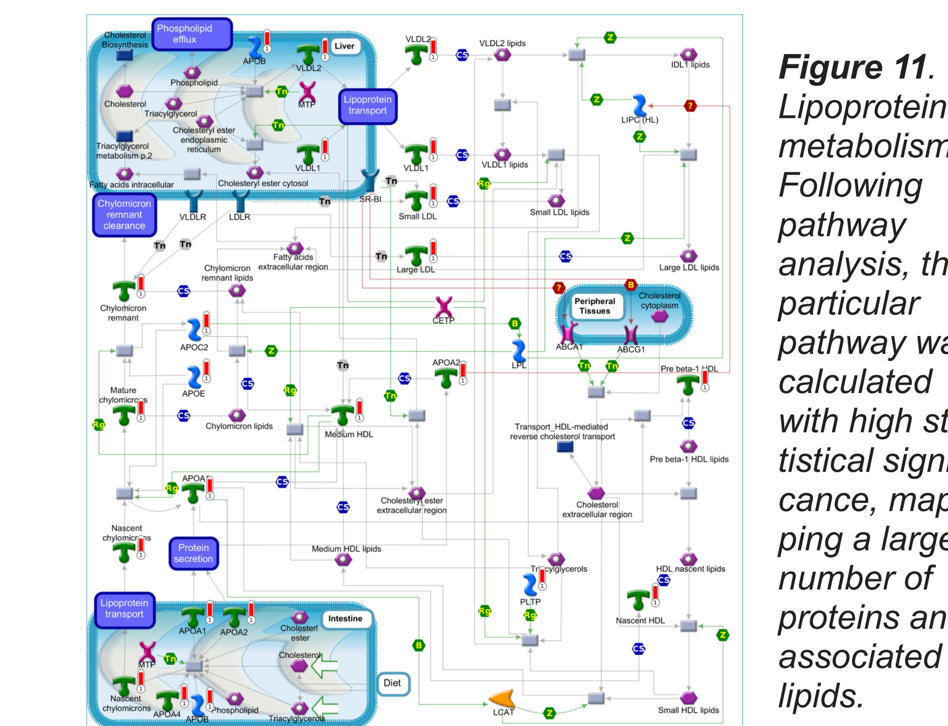


***Figure 11**. Lipoprotein metabolism. Following pathway analysis, this particular pathway was calculated with high statistical significance, mapping a large number of proteins and associated lipids.*

CONCLUSIONS

- **A Multi-omics study, incorporating polar metabolites, lipids and peptides, has been demonstrated whilst using the same LC system**
- **Statistically significant metabolites, lipids and peptides were identified and PCA plots showed reasonable separation between disease states and control / QC**
- **Plasma proteins have been quantified using a stable isotope labelled 'spike in' peptide mixture**
- **Pathway analysis revealed several of significance, including one for lipoprotein metabolism**

References

[1] Richardson *et al*, ASMS 2015
[2] Moseley *et al*, J. Proteome Res., 2018, 17(2), 770-779
[3] Juvvadi *et al*, J. Proteome Res., 2018, 17(2), 780-793

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